

Figure 1

Figure 2

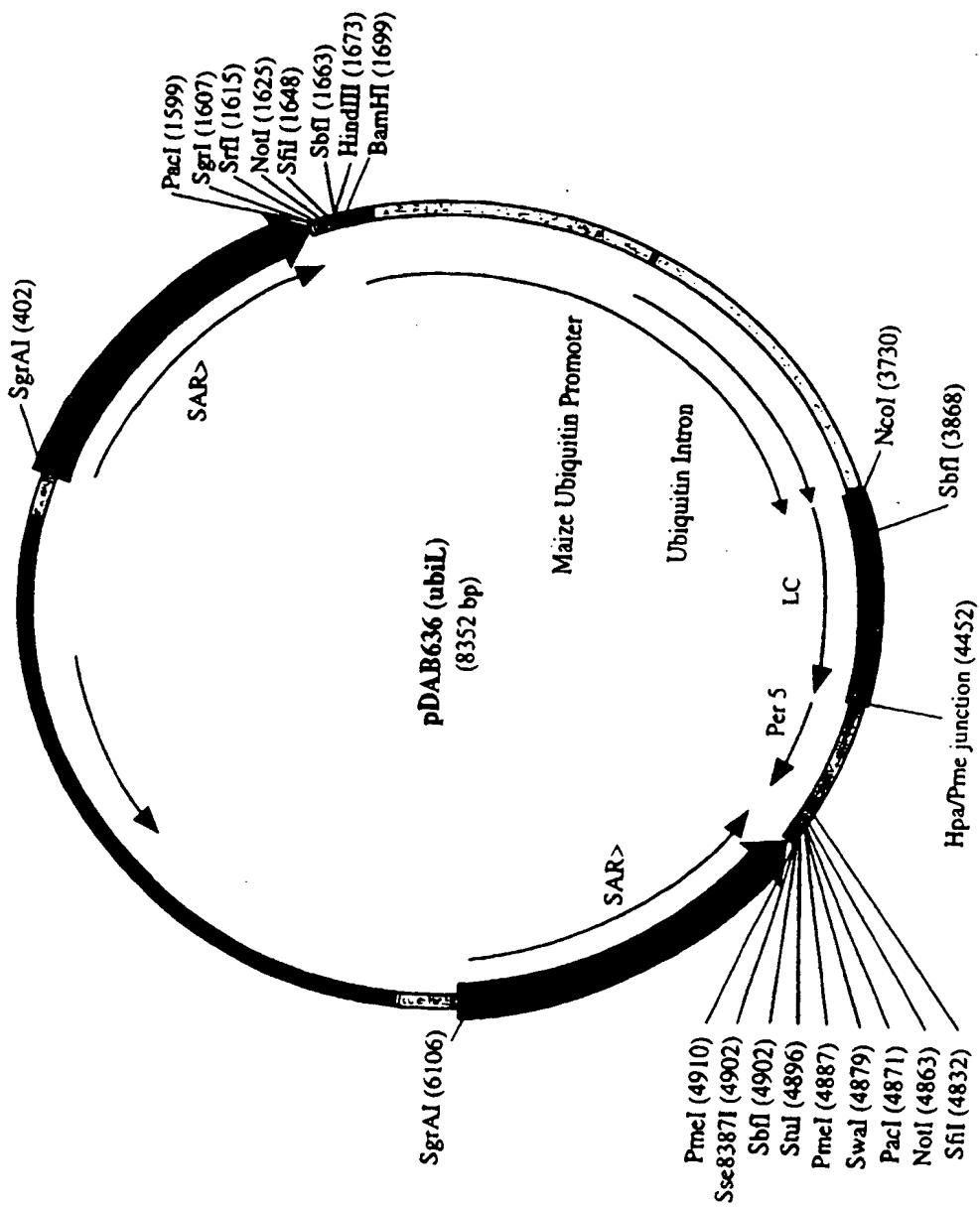
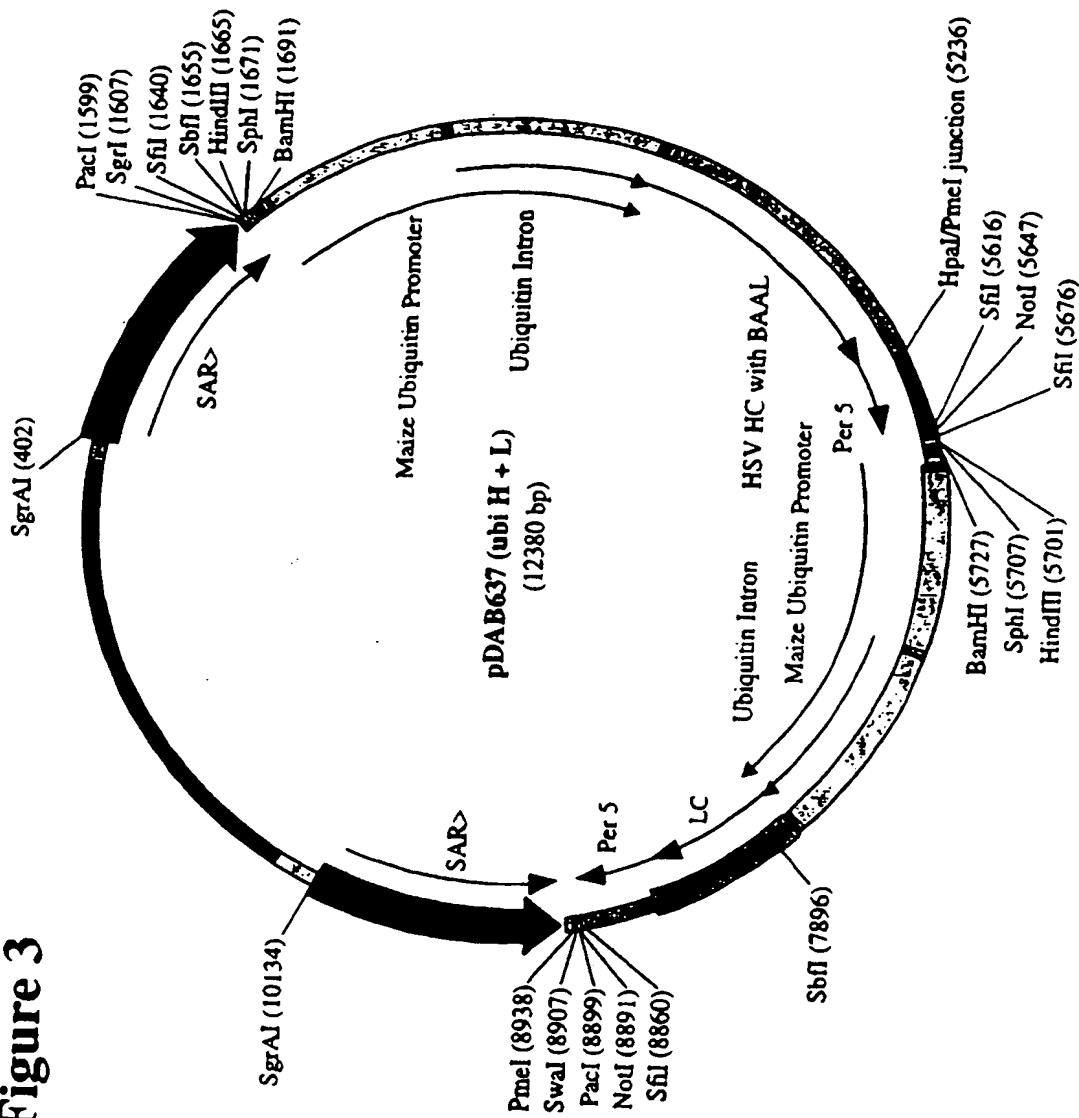


Figure 3



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Figure 4**Native Western Blot (A)**

Western blot condition: 4-12% GEL nonreducing sample buffer
62 ng total protein each well
1:5000 Goat anti-Human Kappa-HRP one hour RT
5 minute expose

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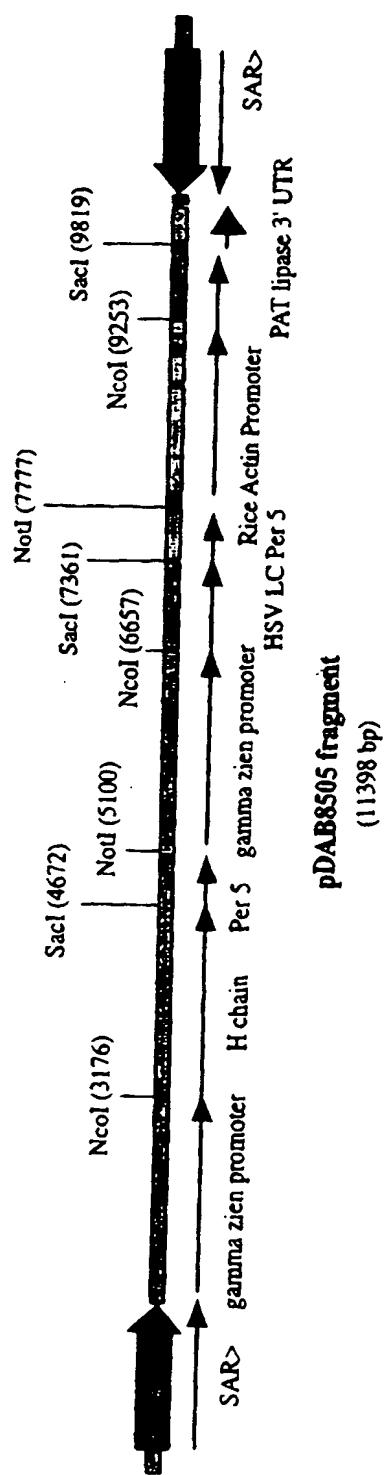


Figure 5

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Figure 6

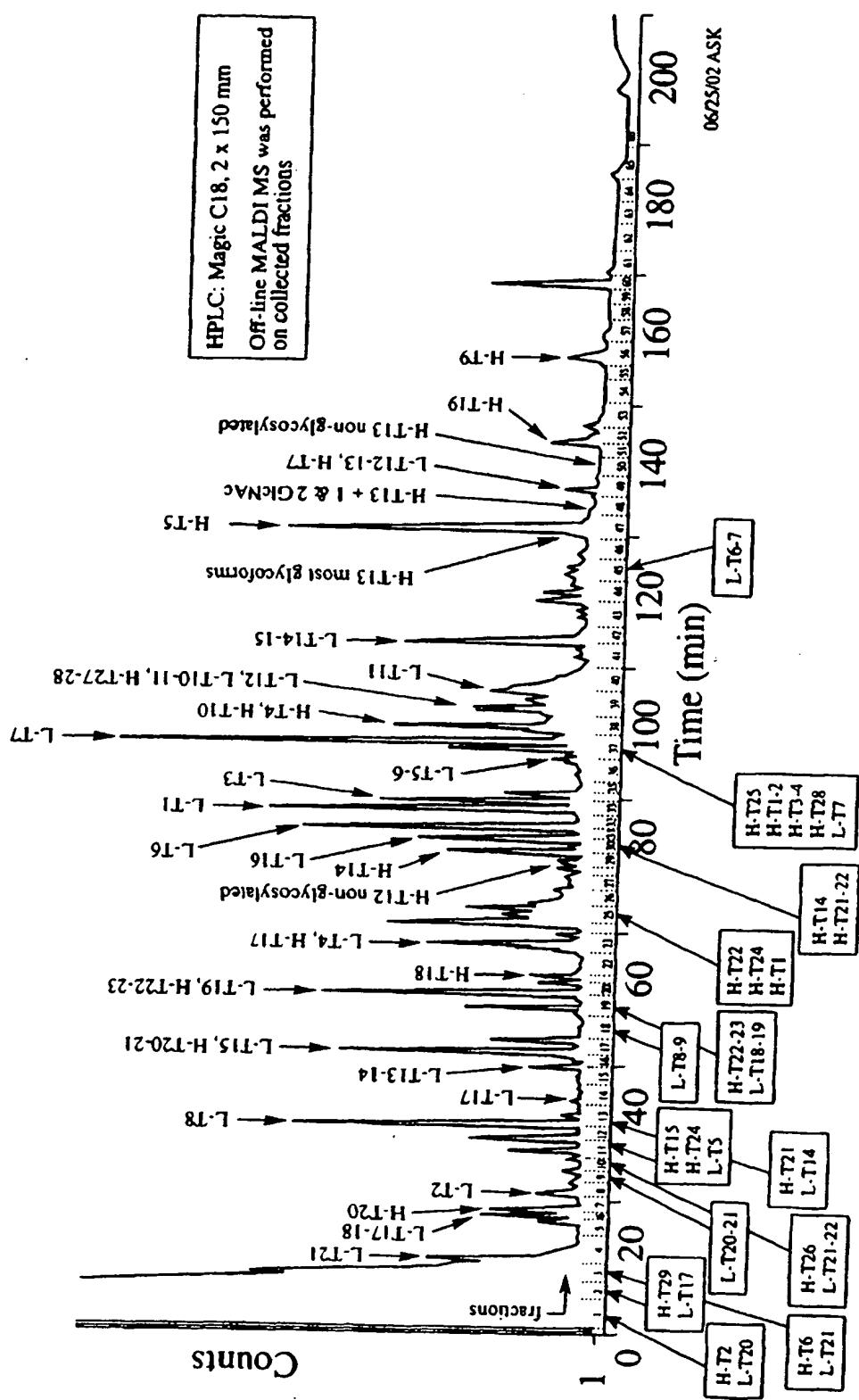


Figure 7

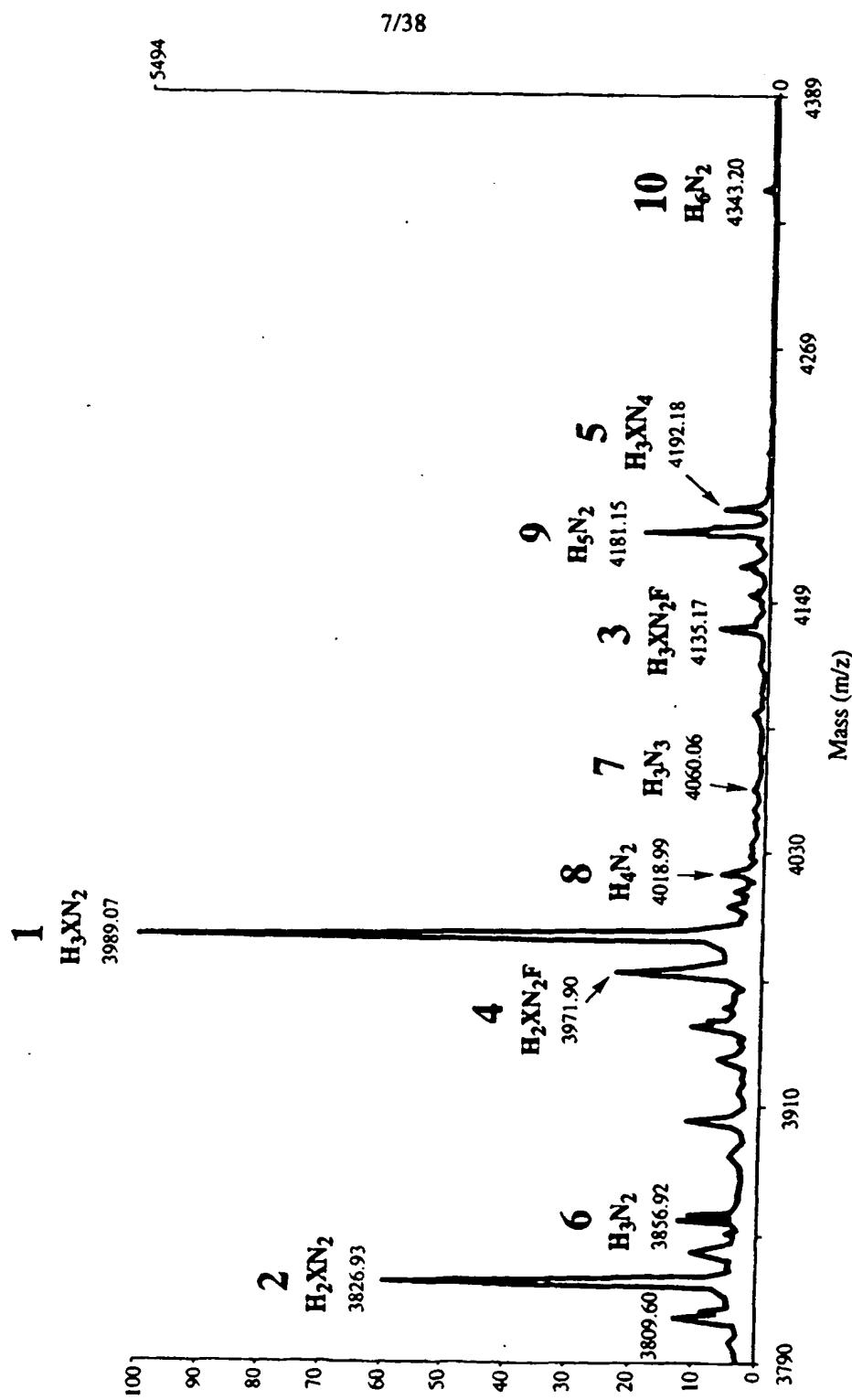


Figure 8

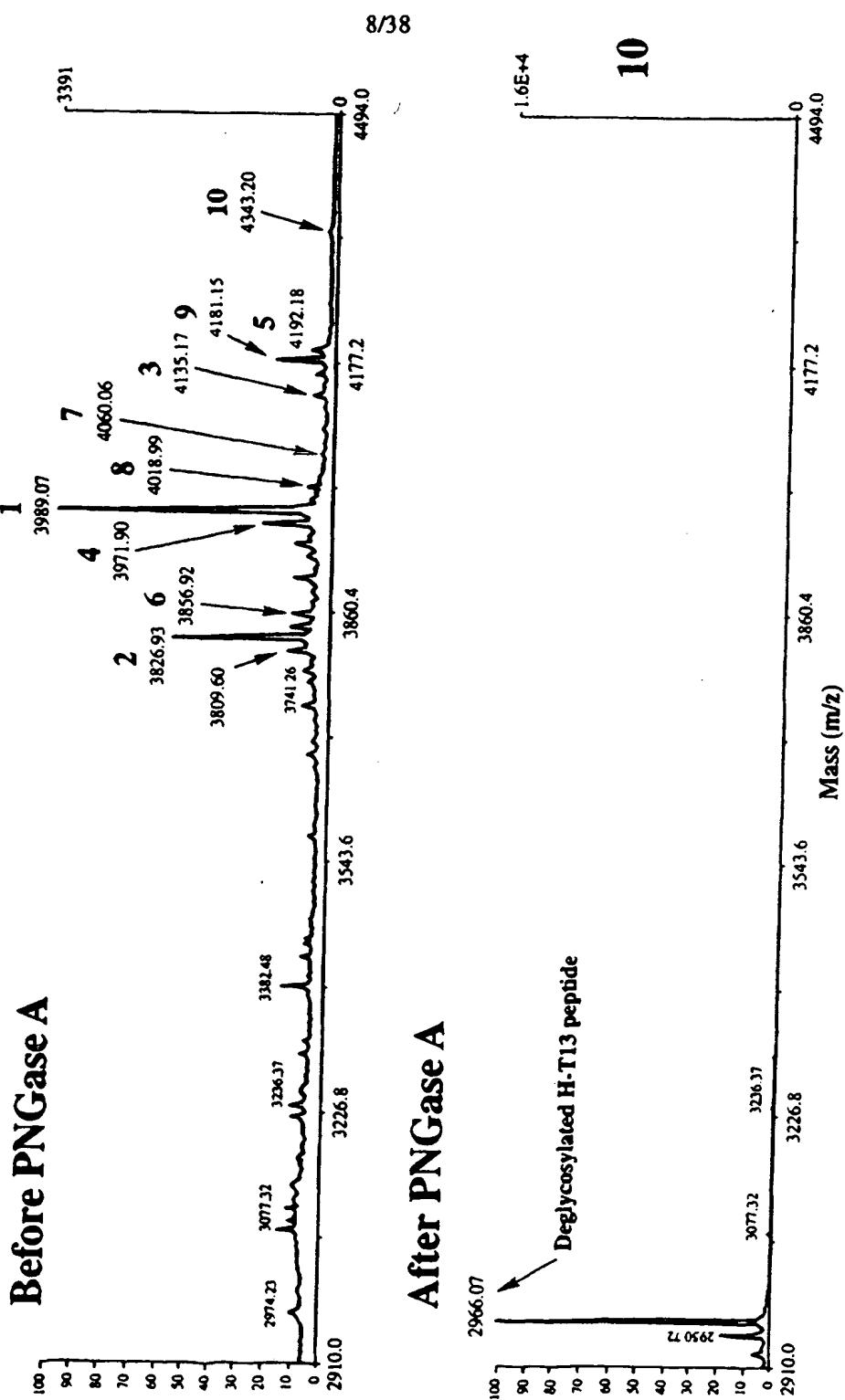
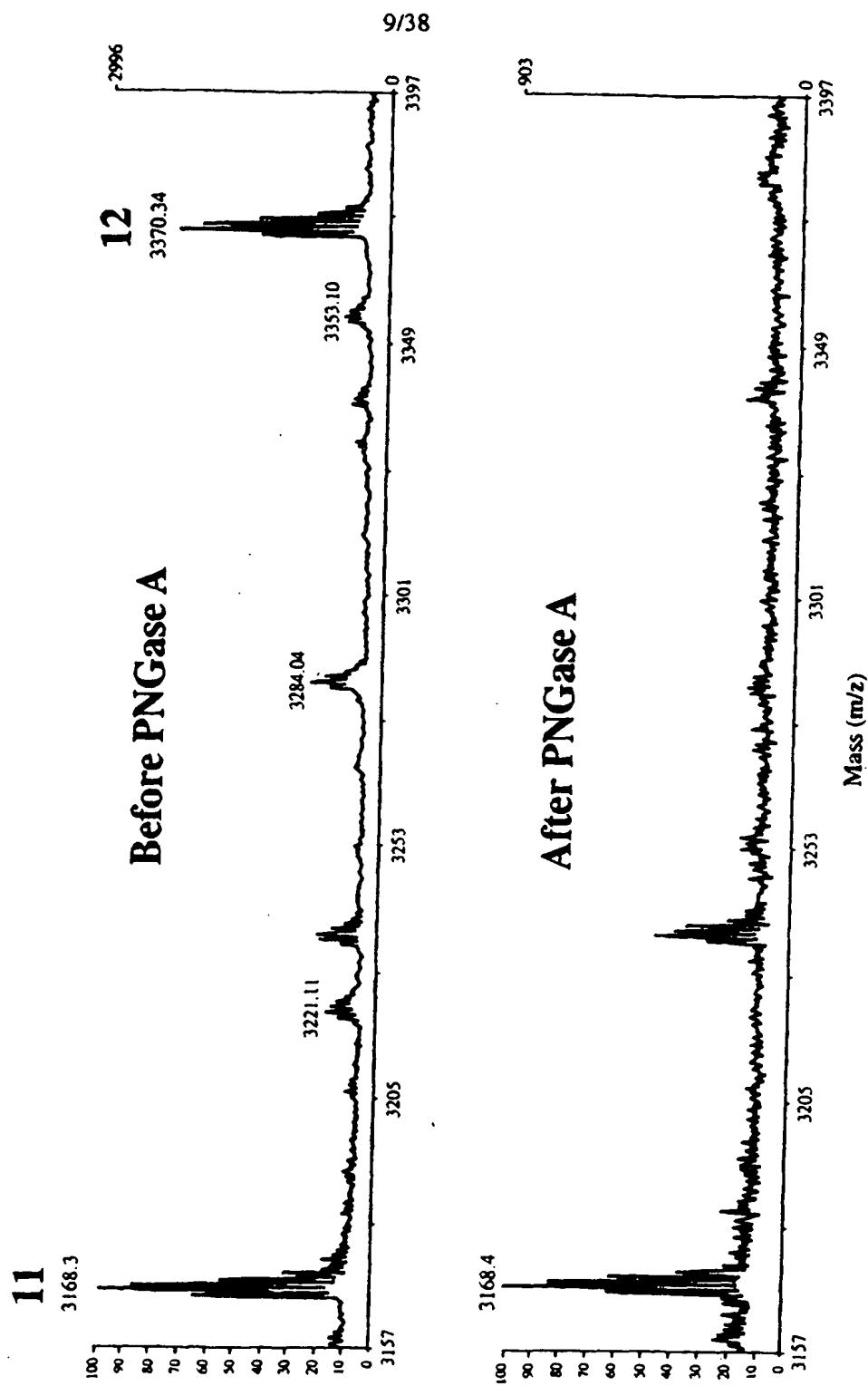
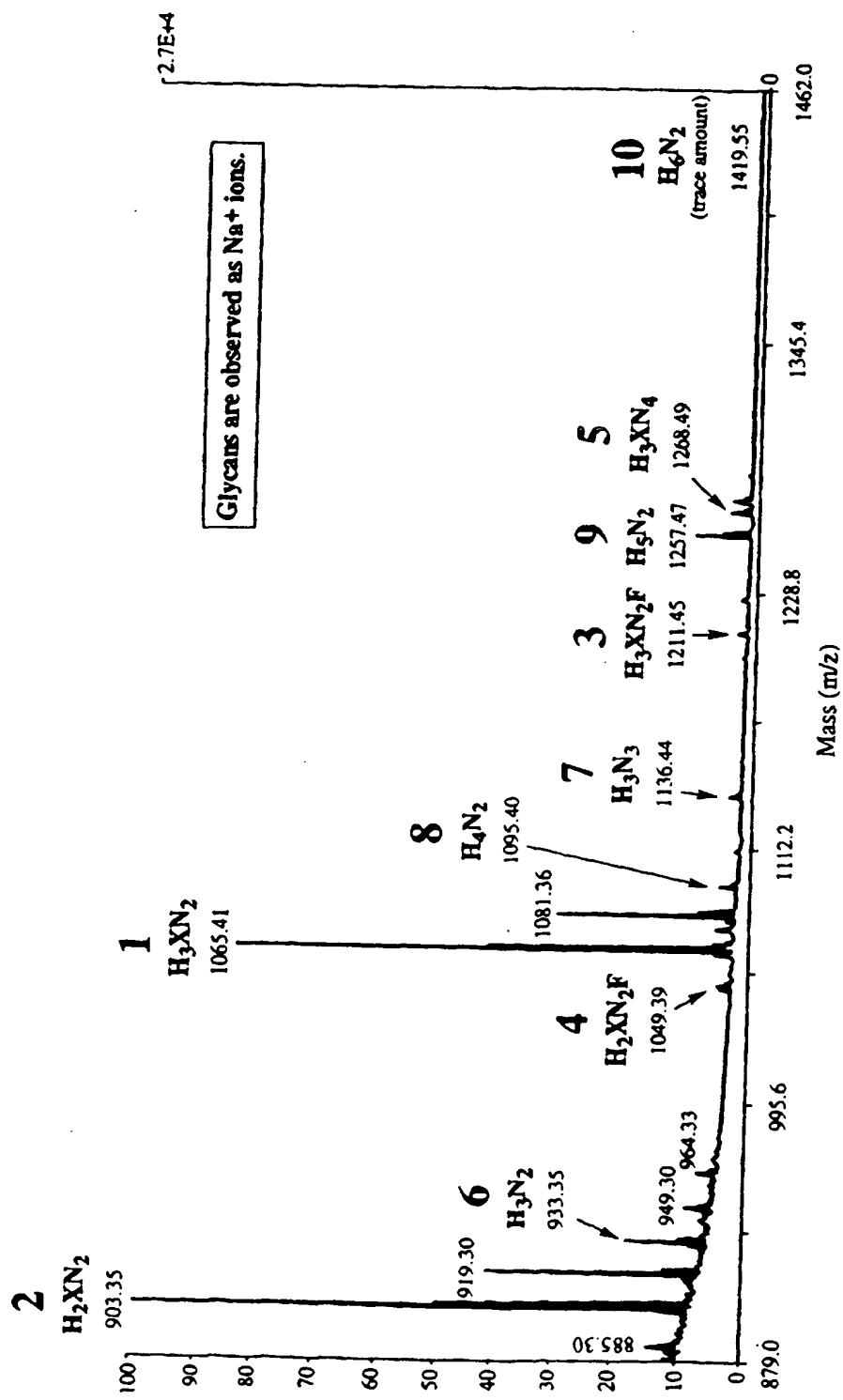


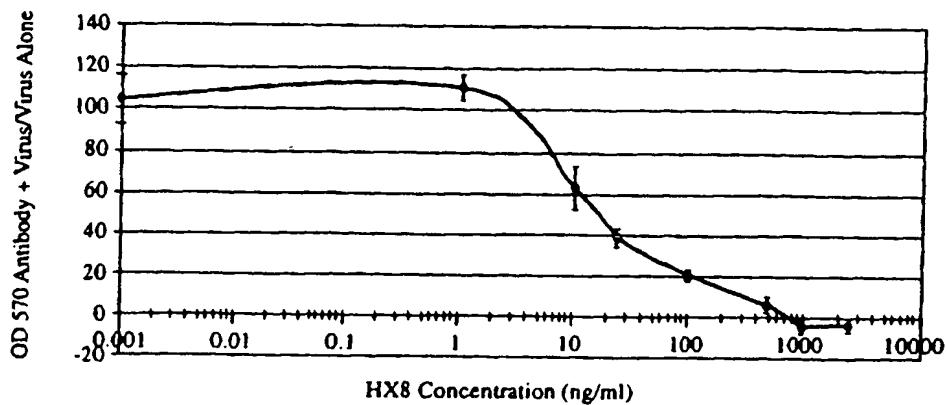
Figure 9

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Figure 10



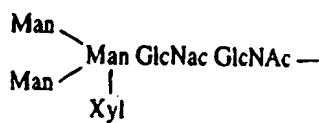
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Figure 11**Neutralization of HSV-2 Using Endosperm Derived HX8**

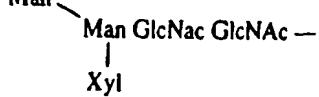
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Figure 12

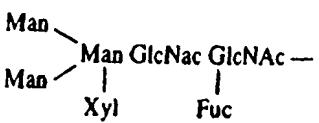
1. 3Man, 2GlcNAc, 1Xyl
~ 38.0 ± 1.8%



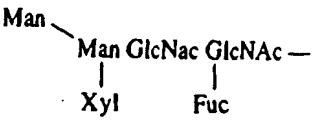
2. 2Man, 2GlcNAc, 1Xyl
~ 42.7 ± 1.3%



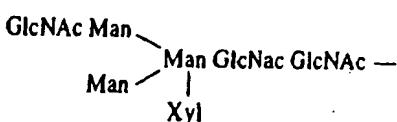
3. 3Man, 2GlcNAc, 1Xyl, 1Fuc
~ 0.9 ± 0.2%



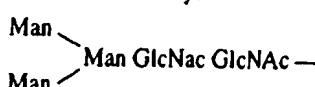
4. 2Man, 2GlcNAc, 1Xyl, 1Fuc
~ 1.3 ± 0.4%



5. 3Man, 3GlcNAc, 1Xyl
~ 1.7 ± 0.1%



6. 3Man, 2GlcNAc
~ 6.2 ± 0.3%



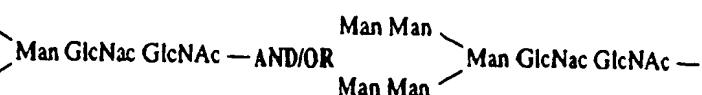
7. 3Man, 3GlcNAc
~ 1.0 ± 0.1%



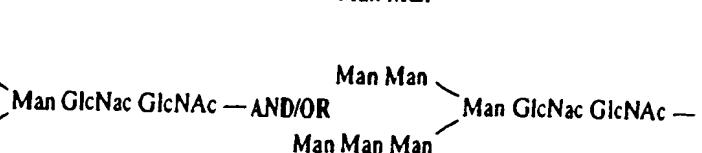
8. 4Man, 2GlcNAc
~ 1.4 ± 0.37%



9. 5Man, 2GlcNAc
~ 4.5 ± 0.2%



10. 6Man, 2GlcNAc
trace



11. 1GlcNAc



12. 2GlcNAc



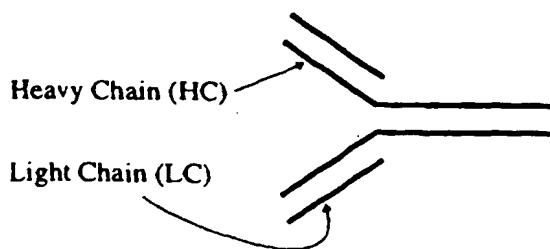
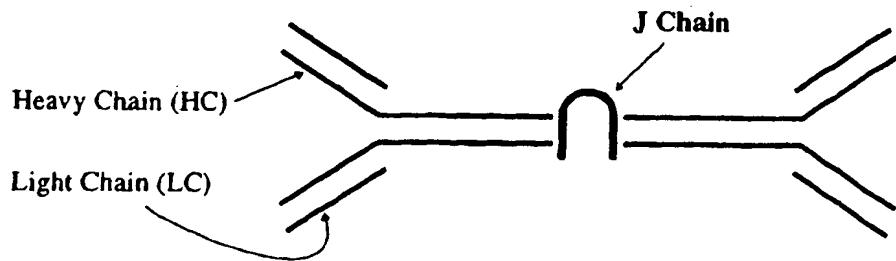
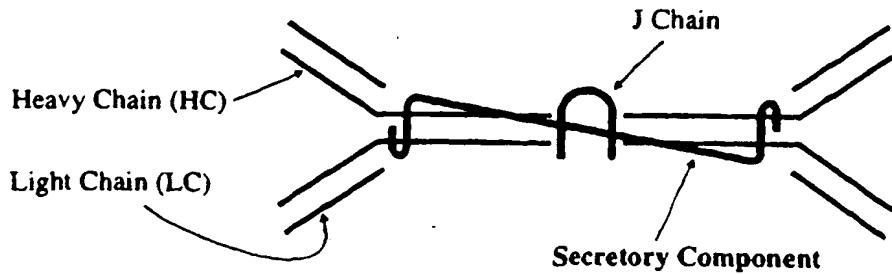
Figure 13A**Monomeric IgA****Figure 13B****Dimeric IgA****Figure 13C****Secretory IgA**

Figure 14

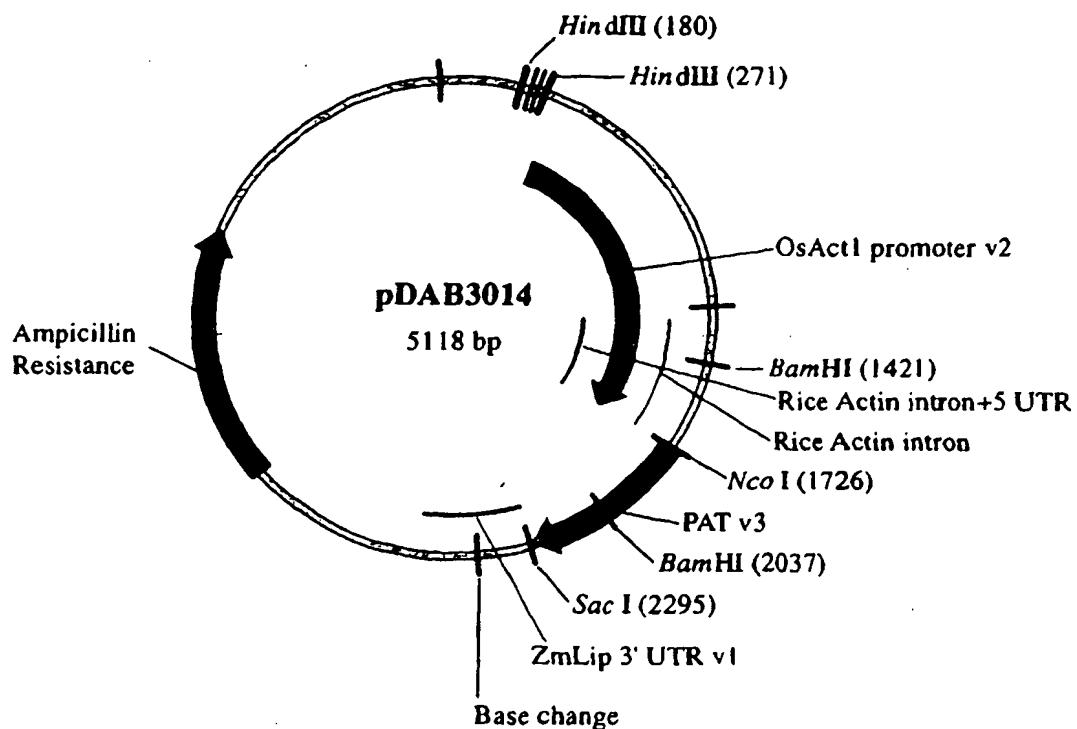


Figure 15

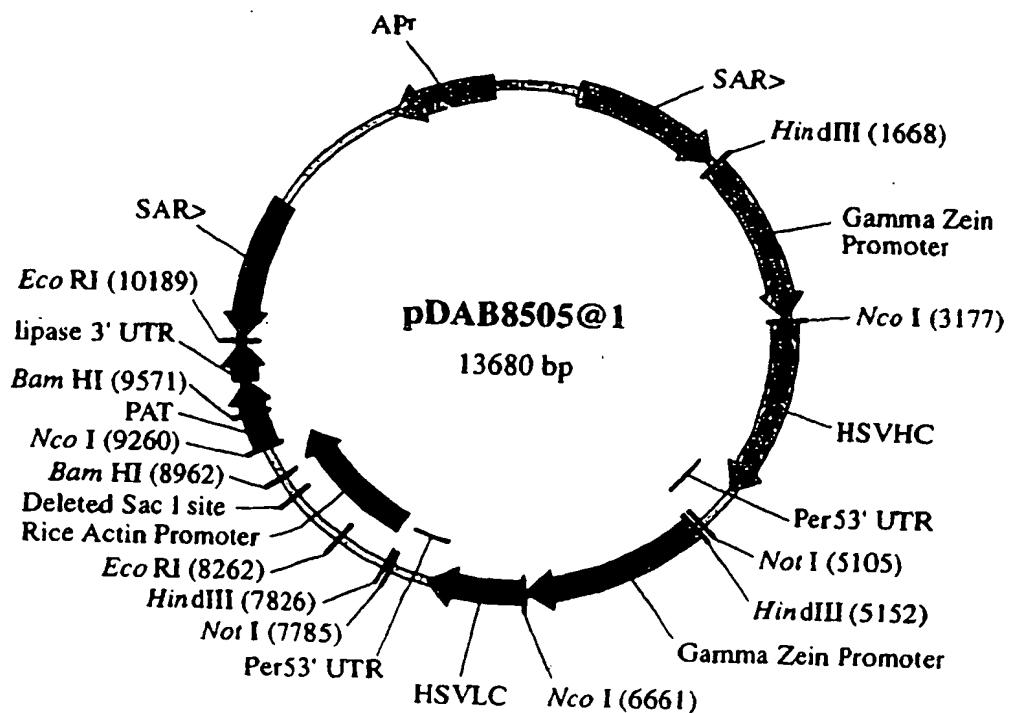


Figure 16A

Event	Glycans				Method of glycan release
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure	
81 (5XH751/ 280-081.005.006)	903.28	903.32	40.6	H2N2X	whole affinity purified IgA-HX8 was digested with pepsin, then PNGase A
	1013.27	1013.29	12.9	H3N2P	
	1065.31	1065.38	25.7	H3N2X	
	1157.29	?	3.3	?	
	1175.29	1179.34	6.5	H4N2P	
	1211.31	1211.44	3.0	H3N2XF	
	ND	1257.46	ND	H5N2	
	1268.37	1268.46	2.1	H3N3X	
	1337.34	1337.40	3.7	H5N2P	
	1499.33	1499.45	1.0	H6N2P	
81 (5XH751/ 280-081.005.006)	1661.36	1661.50	1.1	H7N2P	
	903.5	903.32	20.7	H2N2X	affinity purified IgA-HX8 was separated by SDS-PAGE, band at ~50 kDa was digested in-gel with trypsin, peptides were extracted, purified (C18), then digested with PNGase A
	933.52	933.34	15.3	H3N2	
	1013.52	1013.29	9.9	H3N2P	
	1065.59	1065.38	35.6	H3N2X	
	1157.61	?	12.0	?	
	ND	1211.44	ND	H3N2XF	
	1257.65	1257.46	2.9	H5N2	
	1268.69	1268.46	3.5	H3N3X	

Figure 16B

Event	Glycans				Method of glycan release
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure	
81 (6RC172/ 280-081.005.006)	903.2	903.32	36.9	H2N2X	whole affinity purified IgA-HX8 was digested with pepsin, then PNGase A
	1013.19	1013.29	10.4	H3N2P	
	1065.23	1065.38	28.3	H3N2X	
	1175.24	1175.34	7.3	H4N2P	
	1211.38	1211.44	2.8	H3N2XF	
	1257.27	1257.46	2.8	H5N2	
	ND	1268.46	ND	H3N3X	
	1337.26	1337.40	4.6	H5N2P	
	1499.27	1499.45	3.4	H6N2P	
	1661.36	1661.50	2.0	H7N2P	
81 (6RC172/ 280-081.005.006)	1823.42	1823.55	1.7	H8N2P	affinity purified IgA-HX8 was separated by SDS-PAGE, band at ~50 kDa was digested in-gel with trypsin, peptides were extracted, purified (C18), then digested with PNGase A
	903.51	903.32	21.9	H2N2X	
	933.51	933.34	17.5	H3N2	
	1013.54	1013.29	11.8	H3N2P	
	1065.6	1065.38	32.0	H3N2X	
	1157.61	?	11.1	?	
	1211.66	1211.44	2.5	H3N2XF	
	1257.69	1257.46	3.4	H5N2	
	ND	1268.46	ND	H3N3X	

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Figure 16C

Event	Glycans				Method of glycan release	
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure		
21 self	903.26	903.32	57.4	H2N2X	whole affinity purified IgA-HX8 was digested with pepsin, then PNGase A	
	1013.24	1013.29	10.6	H3N2P		
	1065.3	1065.38	16.2	H3N2X		
	1175.3		4.0	H4N2P		
	1211.36	1211.44	3.8	H3N2XF		
	1257.34	1257.46	1.3	H5N2		
	ND	1268.46	ND	H3N3X		
	1337.37	1337.40	2.7	H5N2P		
	1499.43	1499.45	1.5	H6N2P		
	1661.49	1661.50	1.3	H7N2P		
	1823.54	1823.55	1.0	H8N2P		
21 (5HX751/ 280-021.002.007)	903.17	903.32	36.2	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, then digested with PNGase A and released glycans were analyzed by MALDI	
	933.16	933.34	5.3	H3N2		
	1049.2	1049.38	2.7	H2N2XF		
	1065.2	1065.38	42.8	H3N2X		
	1095.18	1095.40	1.8	H4N2		
	ND	1136.42	ND	H3N3		
	1211.25	1211.44	3.5	H3N2XF		
	1257.25	1257.46	3.2	H5N2		
	1268.26	1268.46	3.6	H3N3X		
	1419.29	1419.52	1.0	H6N2		
	detected on H-T13 glycopeptide		N (single GlcNAc)			
	trace detected on H-T13 glycopeptide		N2 (double GlcNAc)			

Figure 16D

Event	Glycans				Method of glycan release
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure	
21 (6RC172/ 280-021.002.007)	903.12	903.32	42.8	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, then digested with PNGase A and released glycans were analyzed by MALDI
	933.12	933.34	9.3	H3N2	
	1049.14	1049.38	2.8	H2N2XF	
	1065.13	1065.38	35.2	H3N2X	
	1095.13	1095.40	2.2	H4N2	
	1136.16	1136.42	trace	H3N3	
	1211.16	1211.44	2.7	H3N2XF	
	1257.16	1257.46	3.6	H5N2	
	1268.16	1268.46	1.4	H3N3X	
	1419.17	1419.52	trace	H6N2	
detected on H-T13 glycopeptide				N (single GlcNAc)	
trace detected on H-T13 glycopeptide				N2 (double GlcNAc)	

Figure 16E

Event	Glycans				Method of glycan release
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure	
193 self	903.35	903.32	42.7	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, peptides separated by C18-HPLC, HPLC fractions analyzed by MALDI, then fractions containing glycopeptides were digested with PNGase A and released glycans were analyzed by MALDI
	933.35	933.34	6.2	H3N2	
	1049.39	1049.38	1.3	H2N2XF	
	1065.41	1065.38	38.0	H3N2X	
	1095.4	1095.40	1.4	H4N2	
	1136.44	1136.42	1.0	H3N3	
	1211.45	1211.44	0.9	H3N2XF	
	1257.47	1257.46	4.5	H5N2	
	1268.49	1268.46	1.7	H3N3X	
	1419.55	1419.52	trace	H6N2	
detected on H-T13 glycopeptide				N (single GlcNAc)	
detected on H-T13 glycopeptide				N2 (double GlcNAc)	

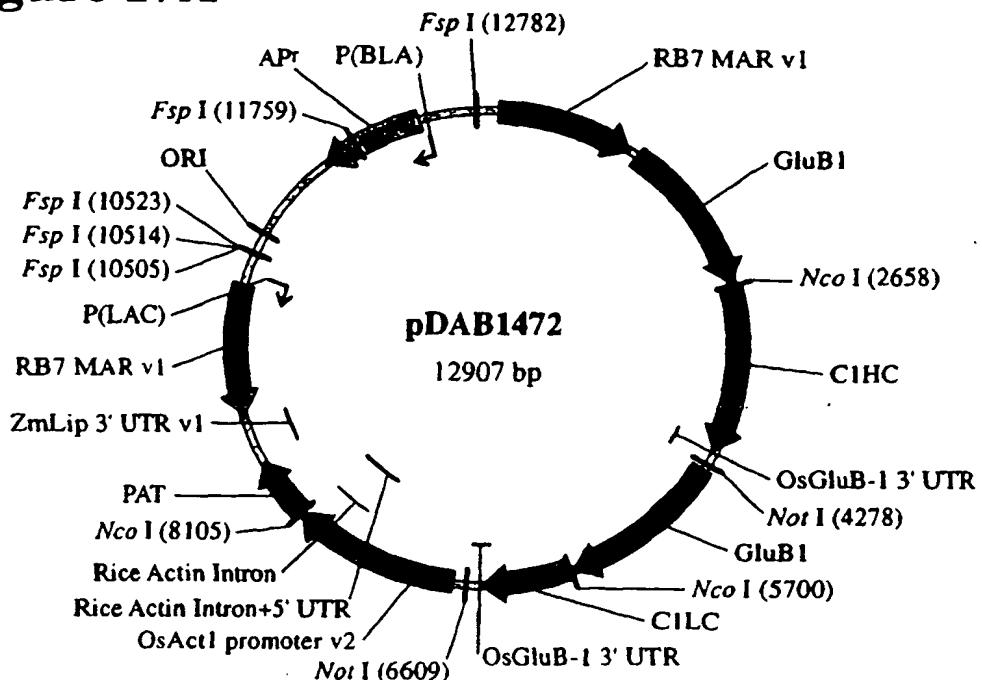
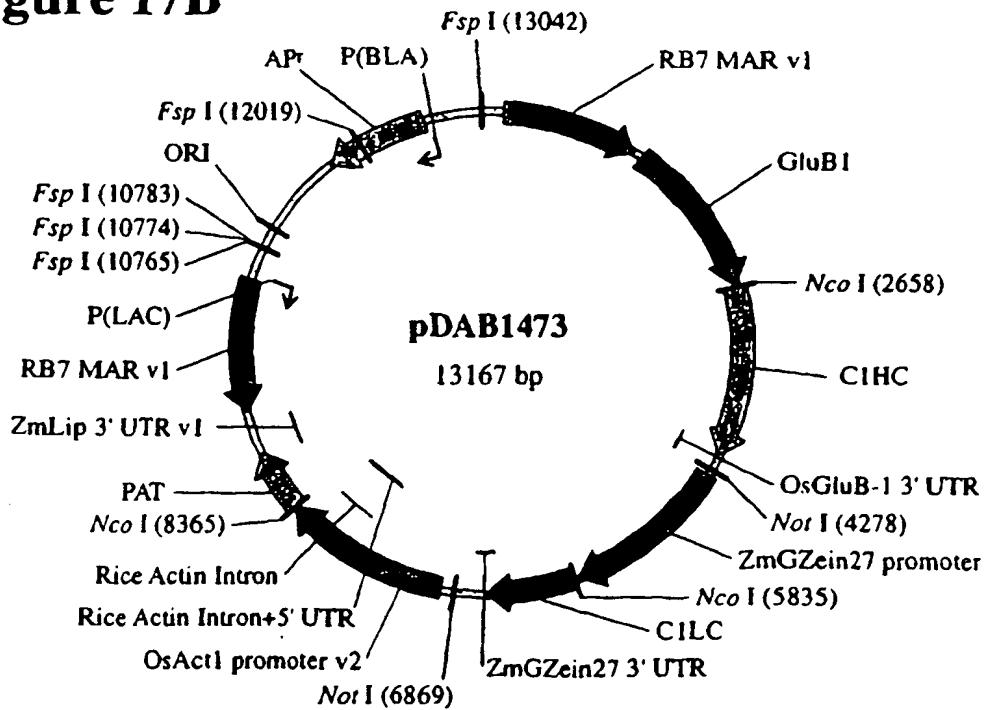
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Figure 16F

Event	Glycans				Method of glycan release	
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure		
223 self	903.51	903.32	34.6	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, then digested with PNGase A and released glycans were analyzed by MALDI	
	ND	933.34	ND	H3N2		
	1065.61	1065.38	55.8	H3N2X		
	1211.68	1211.44	3.9	H3N2XF		
	1257.71	1257.46	5.7	H5N2		
	ND	1268.46	ND	H3N3X		
223 self	903.18	903.32	42.6	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, peptides separated by C18-HPLC. HPLC fractions analyzed by MALDI, then fractions containing glycopeptides were digested with PNGase A and released glycans were analyzed by MALDI	
	ND	933.34	ND	H3N2		
	ND	1049.38	ND	H2N2XF		
	1065.21	1065.38	49.9	H3N2X		
	1095.23	1095.40	trace	H4N2		
	ND	1136.42	ND	H3N3		
	1211.24	1211.44	trace	H3N2XF		
	1257.26	1257.46	7.5	H5N2		
	ND	1268.46	ND	H3N3X		
	ND	1419.52	ND	H6N2		
detected on H-T13 glycopeptide			N (single GlcNAc)			
detected on H-T13 glycopeptide			N2 (double GlcNAc)			

Figure 16G

Event	Glycans				Method of glycan release	
	observed m/z, [M+Na]	Theor. m/z, [M+Na]	% by MALDI (based on peak heights)	Proposed structure		
223 (5HX751/ 280- 223.005.006)	903.28	903.32	40.8	H2N2X	whole affinity purified IgA-HX8 was reduced/ alkylated, digested with trypsin, then digested with PNGase A and released glycans were analyzed by MALDI	
	933.29	933.34	6.1	H3N2		
	1049.33	1049.38	2.0	H2N2XF		
	1065.33	1065.38	40.6	H3N2X		
	1095.33	1095.40	1.3	H4N2		
	1136.4	1136.42	1.2	H3N3		
	1211.37	1211.44	2.8	H3N2XF		
	1257.39	1257.46	2.7	H5N2		
	1268.39	1268.46	2.5	H3N3X		
	1419.12	1419.52	trace	H6N2		
detected on H-T13 glycopeptide			N (single GlcNAc)			
detected on H-T13 glycopeptide			N2 (double GlcNAc)			

Figure 17A**Figure 17B**

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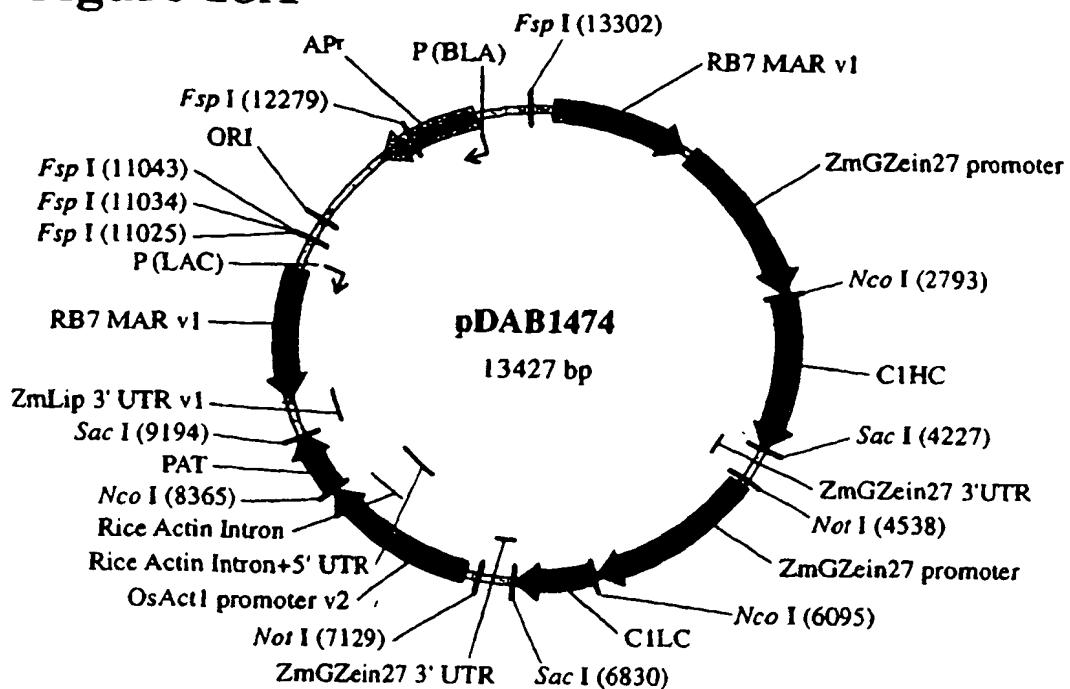
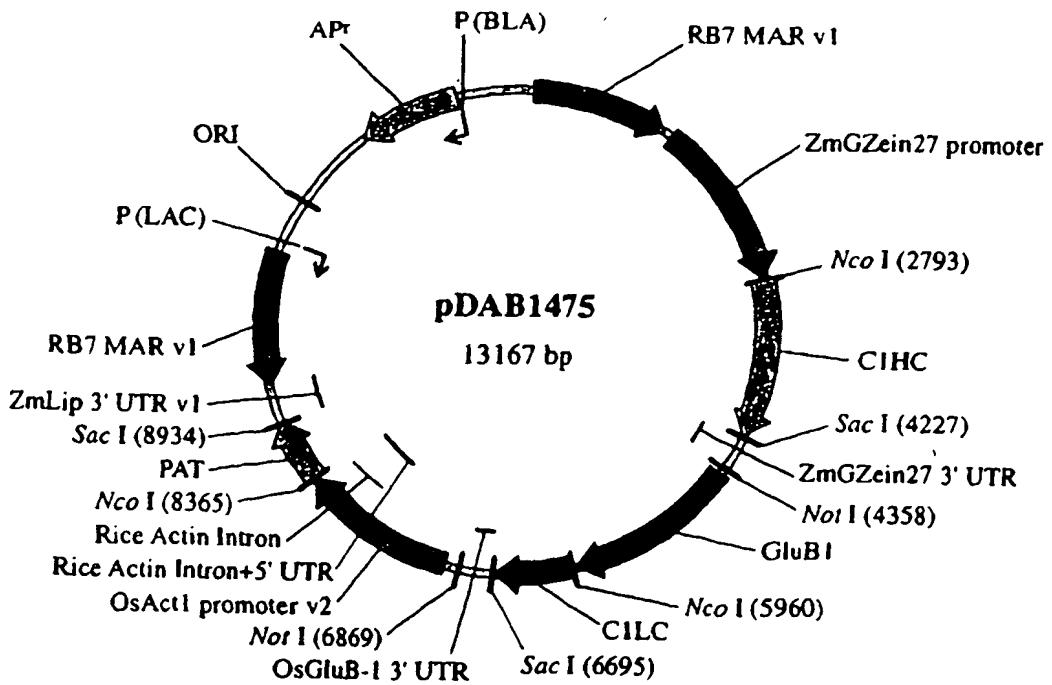
Figure 18A**Figure 18B**

Figure 19

C1-660 IgG

m/z theor.	m/z /obs.	Glycan (glycopeptide)	Comment
1189.51	1189.47	no glycans	
1392.59	1392.54	N	
1595.67	1595.61	N2	
1757.72	1757.83	N2H	
1889.76	ND	N2HX	minor
1903.78	1903.90	N2HXF	ND
1919.77	1919.90	N2H2	trace
2035.82	2035.95	N2HXF	trace
2051.81	2051.95	N2H2X	*
2065.83	2065.96	N2H2F	*
2081.82	2081.95	N2H3	*
2197.87	2197.99	N2H2XF	minor
2213.86	2214.00	N2H3X	major signal
2227.88	2228.01	N2H3F	**
2243.87	2244.02	N2H4	*
2284.90	2285.03	N3H3	*
2359.92	2360.06	N2H3XF	trace
2375.91	2376.07	N2H4X	major signal
2389.93	ND	N2H4F	**
2405.92	2406.07	N2H5	ND
2521.97	2522.16	N2H4XF	significant signal
2537.96	2538.34	N2H5X	*
2551.98	ND	N2H5F	trace
2563.00	2563.16	N3H3XF	ND
2567.97	2568.18	N2H6	significant signal
2684.02	2684.22	N2H5XF	**
2700.01	ND	N2H6X	*
2714.03	ND	N2H6F	ND
2725.05	2725.24	N3H4XF	ND
2730.02	2730.22	N2H7	**
2766.08	2766.26	N4H3XF	**
2892.07	2892.25	N2H8	significant signal
2846.07	ND	N2H6XF	***
2862.06	ND	N2H7X	ND
2876.08	ND	N2H7F	ND
2928.13	2928.32	N4H4F	ND
3008.12	ND	N2H7XF	trace
3054.12	3054.39	N2H9	ND
			trace

NOTE: all these glycans are removed from glycopeptides by PNGase-A treatment; for single N removal is incomplete

Signal intensity: * -- S/N > 3-5, but <10

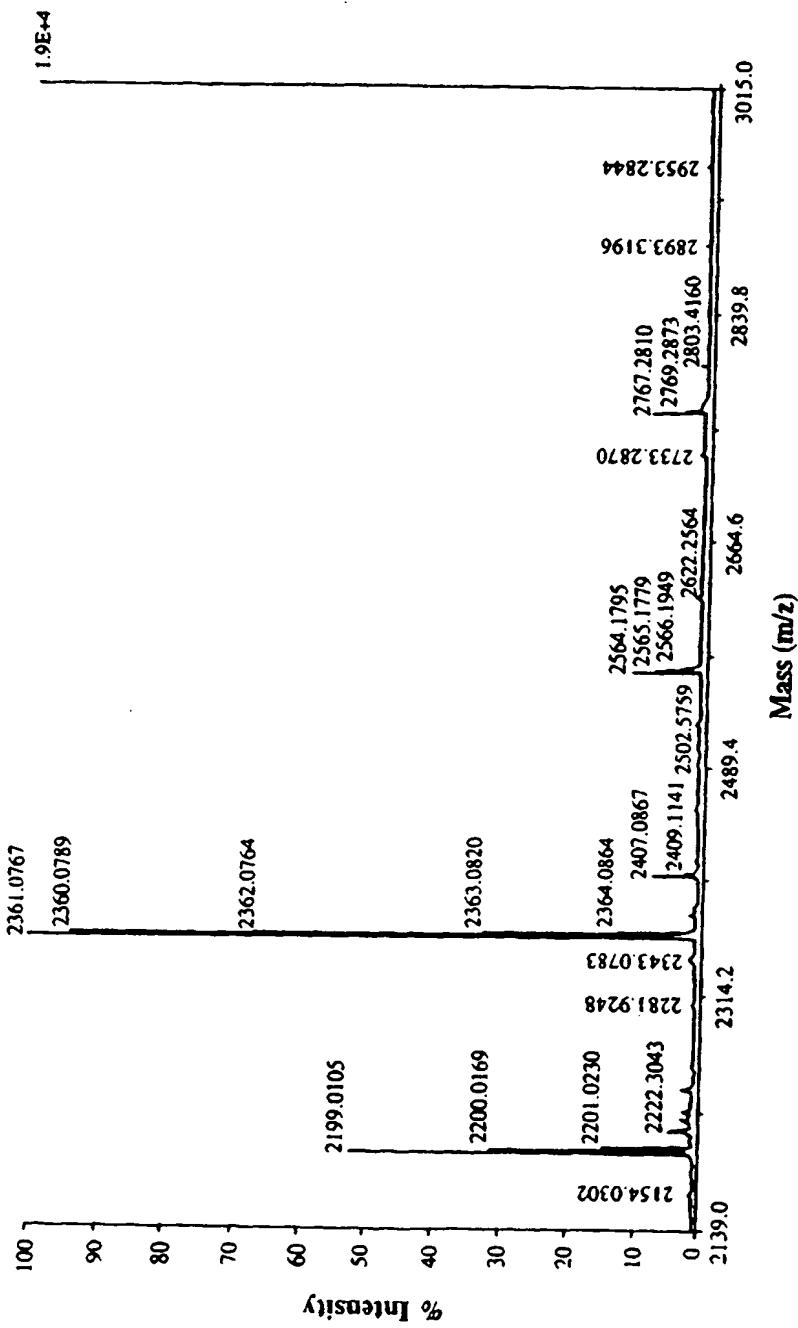
** -- S/N >10

*** -- intense signal, but less intense than "minor"

"significant signal" -- intensity between "minor" and "major"

Figure 20A

Profile of glycoforms of H-T27 peptide (N299 site of heavy chain)
Voyager Spec #1 => BC => SM5 [BP = 560.3, 34501]



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Zoom-in on m/z 2360.06 (major glycoform, N2H3XF). Note isotopic resolution
Voyager Spec #1 => BC => SMS [BP = 560.3, 37865]

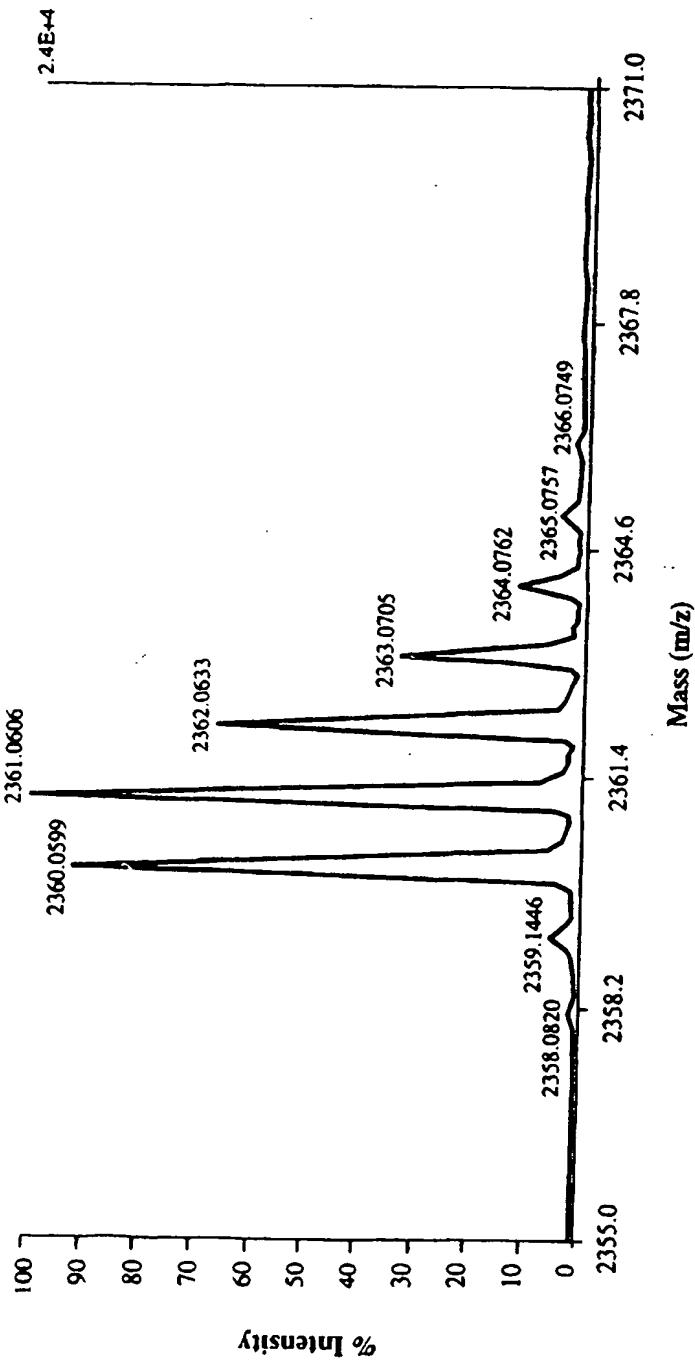


Figure 20B

Figure 21

C1-661 IgG

m/z theor.	m/z /obs.	Glycan (tryptic glycopeptide)	Comment	Corresponding observed free glycan after enzymatic release (see Fig 4)	% of total intensity in MALDI of free glycans
1189.51	1189.61	no glycans	strong signal	n/a	
1392.59	1392.59	N	major signal	n/a	
1595.67	1595.77	N2	*	n/a	
1757.72	1757.89	N2H	trace	ND	
1889.76	ND	N2HX	ND	ND	
1903.78	1903.93	N2HXF	trace	ND	
1919.77	1919.96	N2H2	*	ND	
2035.82	2036.01	N2HXF	*	ND	
2051.81	2052.01	N2H2X	*	ND	
2065.83	2066.03	N2H2F	**	ND	
2081.82	2082.02	N2H3	*	ND	
2197.87	2198.07	N2H2XF	major signal	1049.32 (trace) (1049.38 theor)	trace
2213.86	2214.07	N2H3X	**	1065.34 (1065.38 theor)	24.3
2227.88	2228.09	N2H3F	*	ND	
2243.87	2244.08	N2H4	*	1095.35 (1095.40 theor)	10.5
2284.90	2285.13	N3H3	trace	ND	
2359.92	2360.14	N2H3XF	major signal	1211.40 (1211.44 theor)	31.0
2375.91	2376.14	N2H4X	*	ND	
2389.93	ND	N2H4F	ND	ND	
2405.92	2406.15	N2H5	significant signal	1257.40 (1257.46 theor)	24.6
2521.97	2522.23	N2H4XF	*	ND	
2537.96	ND	N2H5X	ND	ND	
2551.98	ND	N2H5F	ND	ND	
2563.00	2563.25	N3H3XF	significant signal	1414.46 (1414.52 theor)	9.6
2567.97	2568.26	N2H6	**	ND	
2684.02	ND	N2H5XF	ND	ND	
2700.01	ND	N2H6X	ND	ND	
2714.03	ND	N2H6F	ND	ND	
2725.05	2725.32	N3H4XF	*	ND	
2730.02	2730.30	N2H7	**	ND	
2766.08	2766.34	N4H3XF	significant signal	ND	
2892.07	2892.37	N2H8	**	ND	
2846.07	ND	N2H6XF	ND	ND	
2862.06	ND	N2H7X	ND	ND	
2876.08	ND	N2H7F	ND	ND	
2928.13	2928.40	N4H4XF	trace	ND	
3008.12	ND	N2H7XF	ND	ND	
3054.12	ND	N2H9	ND	ND	

Signal intensity: * -- S/N > 3.5, but < 10

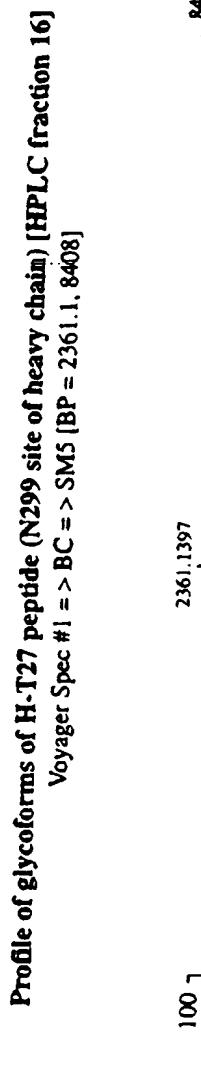
** -- S/N > 10

*** -- intense signal, but less intense than "minor"

"significant signal" -- intensity between "minor" and "major"

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Figure 22A



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Zoom-in on m/z 2360.06 (major glycoform, N2H3XF). Note isotopic resolution.
Voyager Spec #1 => BC => SM5 [BP = 2361.1, 8408]

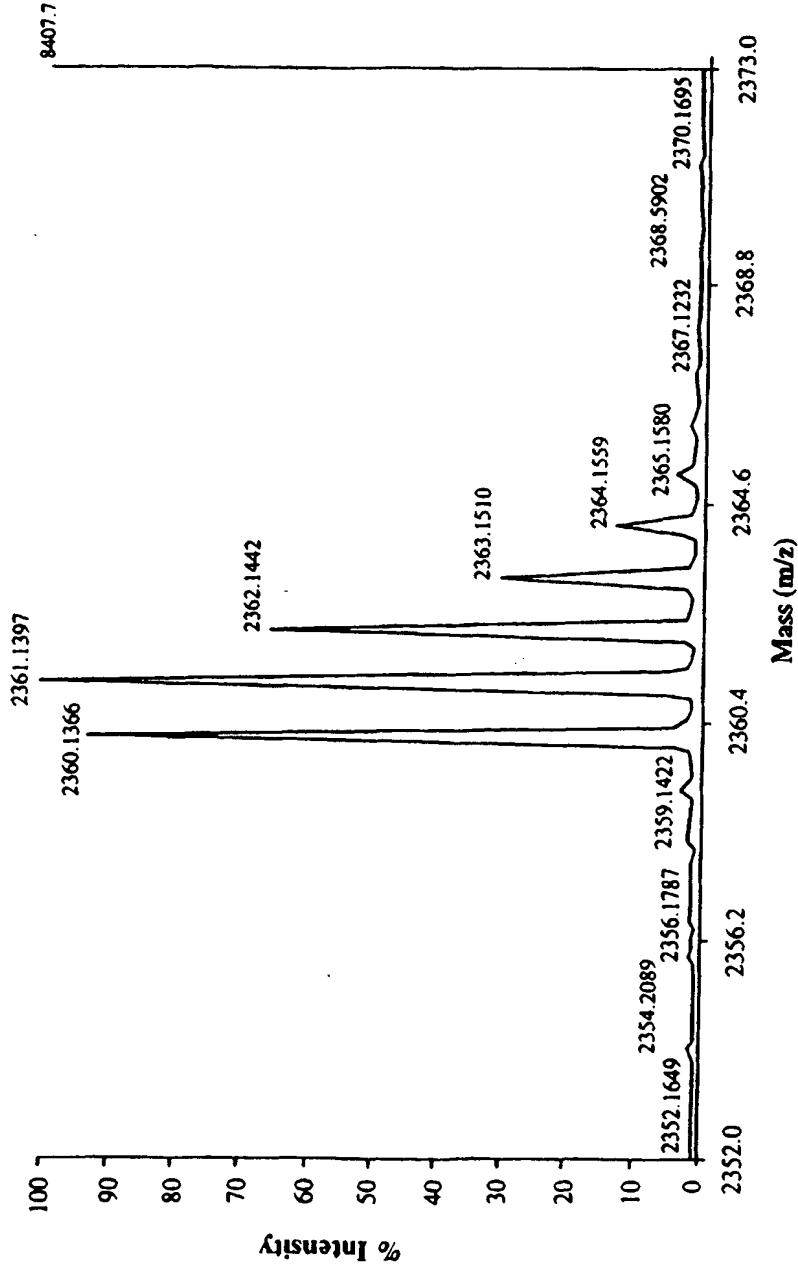
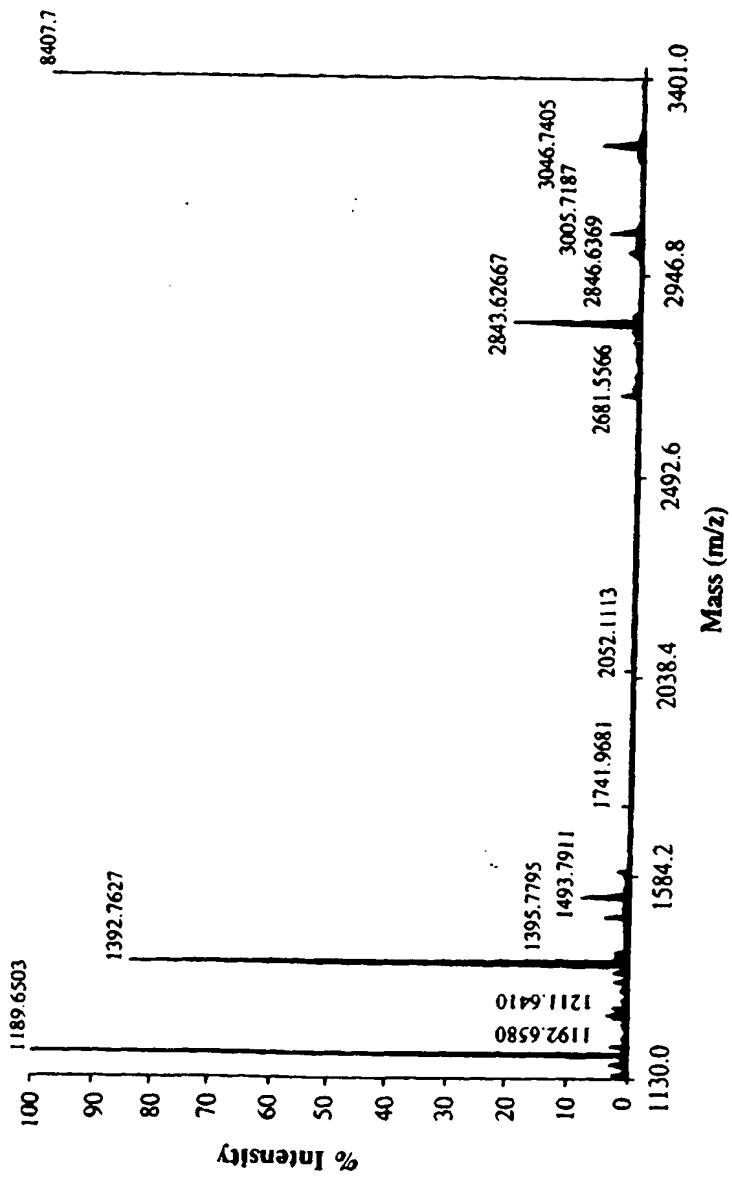


Figure 22B

Figure 22C

H-T27 peptide (non-glycosylated at m/z 1189.65, and with single HexNAc at m/z 1392.76; plus some N-glycoforms on H-T26-27 peptide at higher m/z) [HPLC fraction 17]
Voyager Spec #1 => BC => SMS [BP = 1189.6, 22278]



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Figure 22D

N-glycans released from H-T27 glycopeptide, MALDI MS of free glycans.
 Intensities in this MALDI mass-spectrum should be roughly proportional to abundance
 of the neutral N-glycans (NOTE: single and double GlcNAc are not accounted for.)
 Voyager Spec #1 => BC => SM5 [BP = 1211.4, 1514]

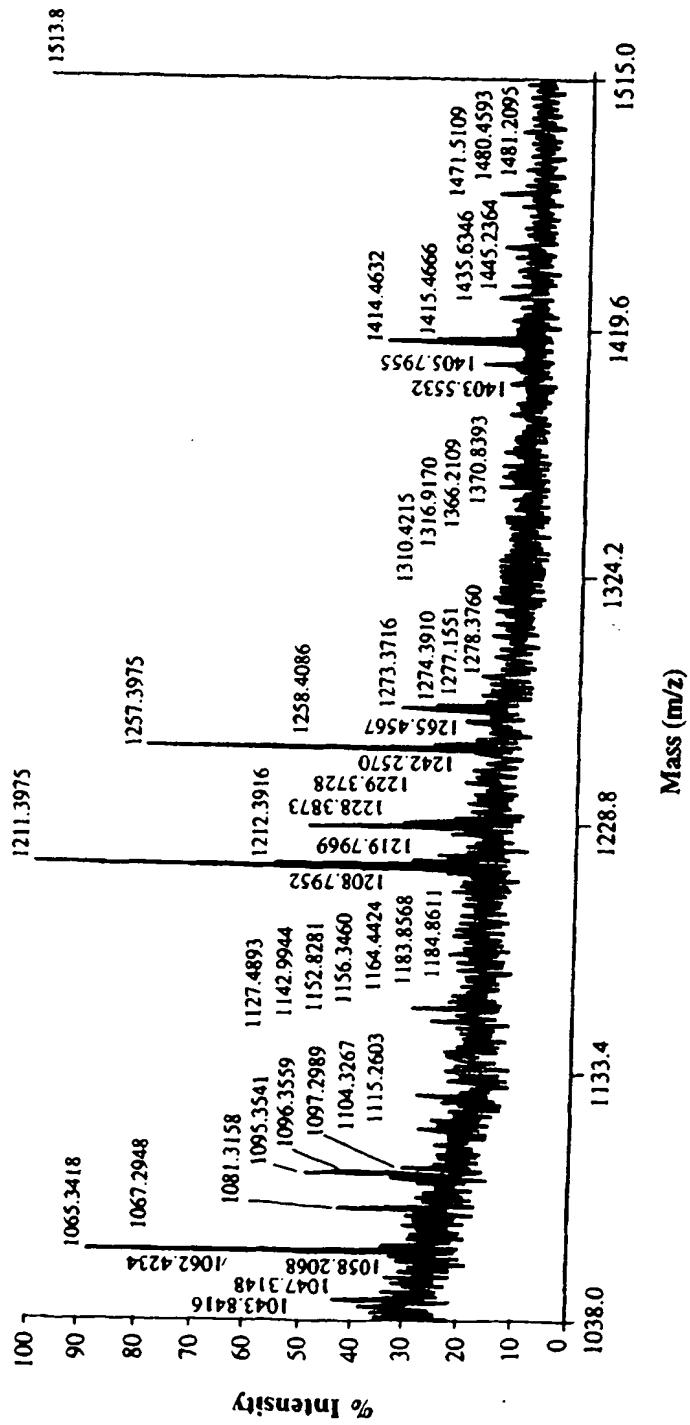


Figure 23

C1-663 IgG

m/z theor.	m/z /obs.	Glycan (glycopeptide)	Comment
1189.51	1189.38	no glycans	Am converts to Am with mass shift of +1 Da after PNGase-A treatment; obs. M/z = 1190.59
1392.59	1392.45	N	major signal
1595.67	1594.72	N2	*
1757.72	1757.84	N2H	trace
1889.76	ND	N2HX	ND
1903.78	ND	N2HXF	ND
1919.77	1919.90	N2H2	trace
2035.82	2035.95	N2HXF	*
2051.81	2051.95	N2H2X	*
2065.83	2065.97	N2H2F	*
2081.82	2081.97	N2H3	*
2197.87	2198.01	N2H2XF	major signal
2213.86	2214.01	N2H3X	**
2227.88	2228.02	N2H3F	*
2243.87	2244.02	N2H4	*
2284.90	ND	N3H3	ND
2359.92	2360.07	N2H3XF	major signal
2375.91	2376.09	N2H4X	*
2389.93	ND	N2H4F	ND
2405.92	2406.08	N2H5	***
2521.97	2522.16	N2H4XF	*
2537.96	ND	N2H5X	ND
2551.98	ND	N2H5F	ND
2563.00	2563.18	N3H3XF	significant signal
2567.97	2568.18	N2H6	**
2684.02	ND	N2H5XF	ND
2700.01	ND	N2H6X	ND
2714.03	ND	N2H6F	ND
2725.05	2725.28	N3H4XF	*
2730.02	2730.24	N2H7	*
2766.08	2766.27	N4H3XF	significant signal
2846.07	ND	N2H6XF	ND
2862.06	ND	N2H7X	ND
2876.08	ND	N2H7F	ND
2892.07	2892.29	N2H8	***
2928.13	ND	N4H4XF	ND
3008.12	ND	N2H7XF	ND
3054.12	ND	N2H9	ND

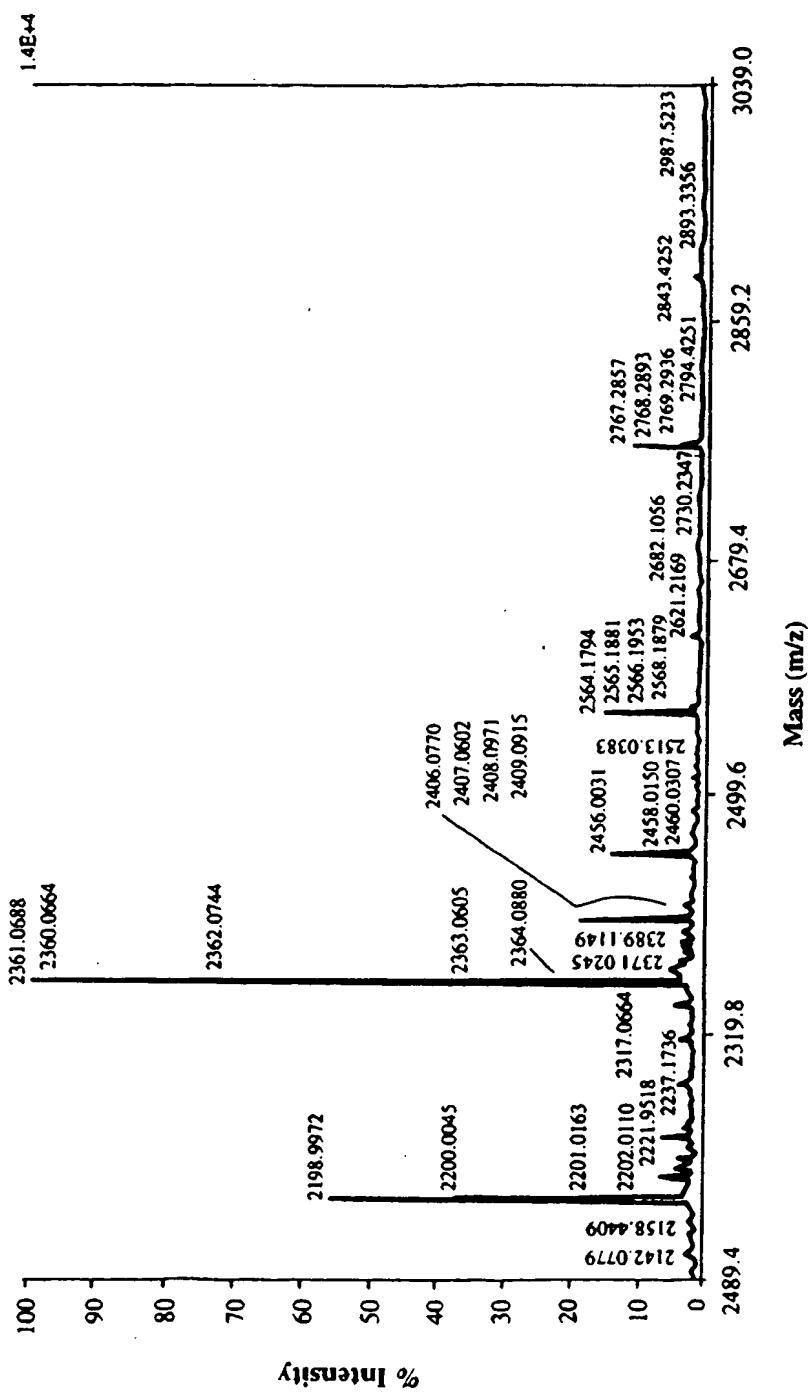
NOTE: all these glycans are removed from glycopeptides by PNGase-A treatment; for single N removal is incomplete

Signal intensity: * -- S/N > 3-5, but <10
 ** -- S/N >10
 *** -- intense signal, but less intense than "minor"
 "significant signal" -- intensity between "minor" and "major"

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Figure 24A

Profile of glycoforms of H-T27 peptide (N299 site of heavy chain)
 Voyager Spec #1 => BC => SMS [BP = 2361.1, 13642]



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Figure 24B

Zoom-in on m/z 2360.7 (major glycoform, $N2H3XF$). Note isotopic resolution.
Voyager Spec #1 => BC => SM5 [BP = 2361.1, 13642]

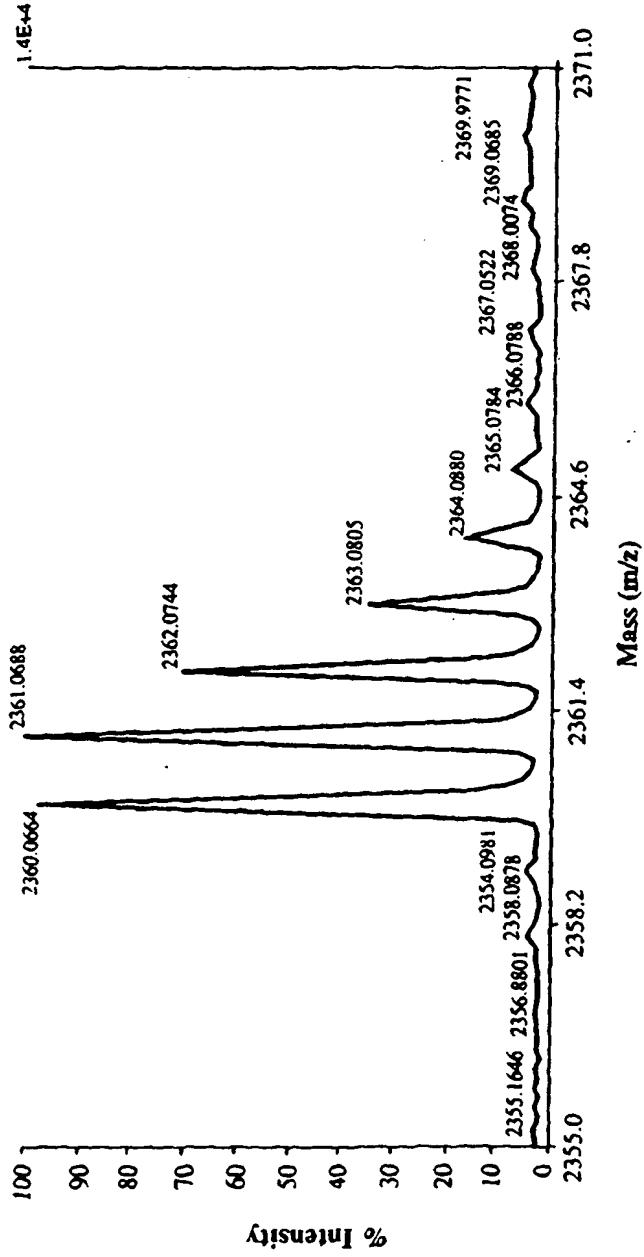


Figure 25

(CHO-expressed)

m/z theor.	m/z /obs.	Glycan (glycopeptide)	Comment
1189.51	1189.63	no glycans	Aza converts to Asp with mass-shift of +1 Da after PNGase-A treatment; obs. M/z = 1190.48
1392.59	1392.74	N	**
1595.67	1595.38	N2	*
1757.72	1757.41	N2H	*
1919.77	1919.44	N2H2	*
2065.83	2065.48	N2H2F	*
2081.82	2081.48	N2H3	*
2227.88	2227.51	N2H3F	*
2243.87	2243.51	N2H4	*
2268.91	2268.52	N3H2F	significant signal
2284.90	2284.52	N3H3	**
2389.93	2389.54	N2H4F	*
2405.92	2405.52	N2H5	significant signal
2430.96	2430.55	N3H3F	significant signal
2446.95	2446.55	N3H4	*
2487.98	2487.56	N4H3	significant signal
2551.98	2552.57	N2H5F	trace
2567.97	2567.57	N2H6	*
2593.01	2592.60	N3H4F	minor
2609.00	2608.60	N3H5	**
2634.04	2633.61	N4H3F	major signal free glycan also observed after release (MNa ⁺ = 1485.87)
2650.03	2649.58	N4H4	minor
2714.03	ND	N2H6F	ND
2730.02	2730.61	N2H7	trace
2755.06	2754.64	N3H5F	**
2771.05	2770.64	N3H6	**
2796.09	2795.64	N4H4F	major signal free glycan also observed after release (MNa ⁺ = 1647.96)
2812.08	2811.63	N4H5	trace
2876.08	ND	N2H7F	ND
2892.07	ND	N2H8	ND
2933.10	2932.67	N3H7	*
2958.14	2957.68	N4H5F	significant signal
3054.12	ND	N2H9	ND
3120.19	3119.73	N4H6F	minor
3282.24	3281.76	N4H7F	***

NOTE: glycans are removed from glycopeptides by PNGase-A treatment; for single N removal is incomplete

Signal intensity: * -- S/N > 3-5, but <10

** -- S/N > 10

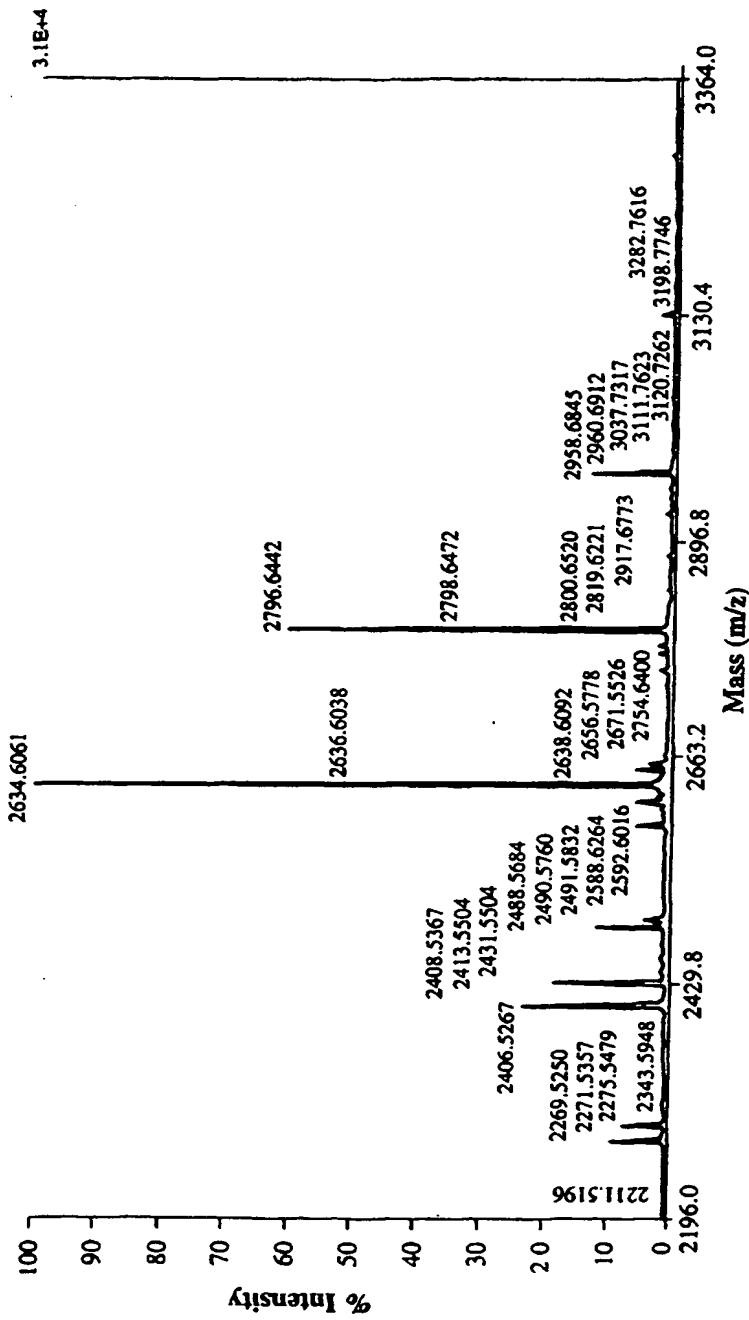
*** -- intense signal, but less intense than "minor"

"significant signal" -- intensity between "minor" and "major"

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Figure 26A

Profile of glycoforms of H-T27 peptide (N299 site of heavy chain)
Voyager Spec #1 => BC => SMS [BP = 2634.6, 30912]



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Zoom-in on m/z 2633.61 (major glycoform, N4H3F). Note isotopic resolution.
Voyager Spec #1 => BC => SMS [BP = 2634.6, 30912]

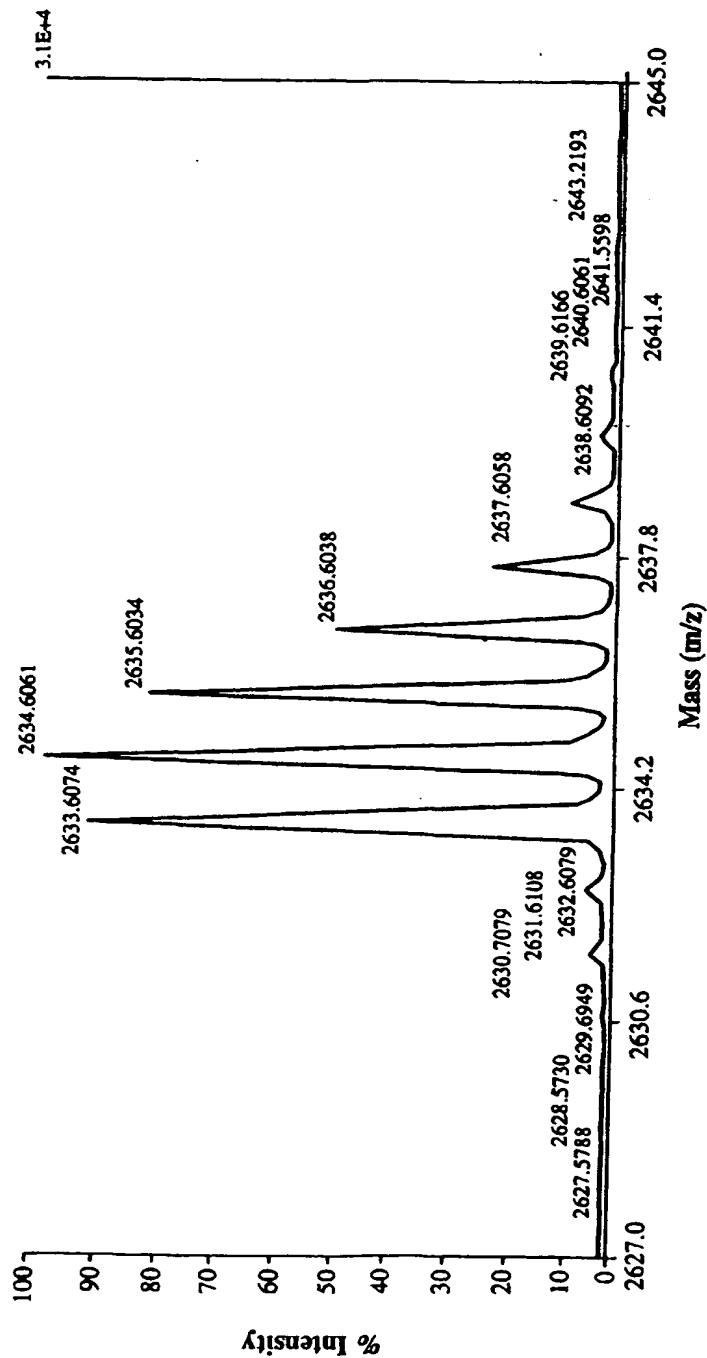


Figure 26B